IN THE UNITED STATES DISTRICT COURT
FOR THE NORTHERN DISTRICT OF OKLAHOMA

STATE OF OKLAHOMA,	)	
	)	
Plaintiff,	)	
	)	
v.	)	**Case No. 05-cv-329-GKF(PJC)**
	)	
TYSON FOODS, INC., et al.,	)	
	)	
Defendants.	)	

DECLARATION OF JENNIFER L WEIDHAAS, Ph.D., P.E.

I, JENNIFER L WEIDHAAS, Ph.D., P.E., declare as follows:

1. My education includes a Bachelor's degree in Civil Engineering with an emphasis in Bio-resource Technology from Montana State University at Bozeman (1999), a Masters in Civil and Environmental Engineering from University of California at Davis (2002) and a Doctorate in Philosophy from University of California at Davis in Civil and Environmental Engineering with a designated emphasis in Biotechnology (2006). I have worked for North Wind Inc., since 2005 as a research scientist, engineer and human health and ecological risk assessor. Prior to working at North Wind I worked as an engineer and risk assessor for the US Department of Energy's Idaho National Laboratory, periodically from 1997 to 1999 and during 2000. I am a registered Professional Engineer in the State of Idaho (License #12525) in the discipline of Environmental Engineering.

2. North Wind, Inc. is an award winning, woman owned small business. North Wind has been named as the "Top Small Business in the state of Idaho" and ranked number 40 of 500 small businesses across the United States by Diversity Business.com. Additionally, North Wind has recently been awarded the EPA Region 10 Subcontractor of the Year award for 2008 by the Small Business Administration. North Wind's owner Sylvia Medina was honored in 2007 with a "Women Impacting Public Policy Fast Five Award." In 2005 the U.S. Small Business Administration honored North Wind Inc. as a USEPA Region 10 Prime Contractor of the Year Award and as one of the top five Women-Owned Businesses in the USA. Additionally the U.S. Small Business Administration honored North Wind with the Administrator's Award of Excellence in 2000, 2001, 2002 and 2005. With more than 350 scientific, engineering, construction, management and professional personnel in 19 offices throughout the country. North Wind Inc. routinely performs molecular analysis of samples including all of the methods used in the identification and development of an assay for a poultry litter associated biomarker. In fact, North Wind Inc. has analyzed thousands of samples by PCR and qPCR since the formation of its molecular laboratory located at Idaho State University.

3. Through my formal training in graduate school and at North Wind I have routinely worked with all the methods used to identify the poultry waste associated biomarker and development of the biomarker assay. North Wind, Inc. was retained to work with Dr. Harwood to determine if there was a poultry litter specific genetic biomarker from poultry feces, and, if so, develop a poultry waste specific biomarker assay to ID and quantify that biomarker. I was responsible for the identification of the

biomarker and development of the assay in collaboration with Drs. Jody Harwood and Tamzen Macbeth. I have reviewed the "DEFENDANTS' MOTION TO EXCLUDE THE TESTIMONY OF DR. VALERIE J. HARWOOD PURSUANT TO DAUBERT v. MERRELL PHARMACEUTICALS, INC." and associated exhibits in addition to Dr. Samuel Myoda's Expert Report and his associated considered materials.

4. The methodology used to identify a poultry litter associated biomarker is not new or novel. Rather it is the application of accepted methods for identifying a microorganism that is associated with poultry litter. A general protocol for developing new Microbial Source Tracking (MST) methods has been published and is presented in Figure 1 shown below and was the basis of the poultry biomarker studies conducted by North Wind in collaboration with Dr. Harwood (Field, KG, M. Samadpour, 2007, Review Fecal source tracking, the indicator paradigm, and managing water quality, Water Research, 41: 3517 – 3538). Specifically the stages of methodology development are described as follows in Fields and Samadpour, 2007:

> *During its development, a new method of fecal source tracking goes through specific stages of testing... The first stage of testing is "proof of concept" testing: the method's ability to classify the samples used in its development is tested in the laboratory. The second stage can be termed "feasibility and biological likelihood" testing: the method is applied in the field to samples of unknown composition in an area where a particular source of contamination is thought to occur (e.g., near a sewage outfall, dairy, or pig farm). If the method can detect its target under field conditions and concentrations, it is feasible; if it detects the likely source, this is taken as proof of biological likelihood. A third stage can be termed "applicability to novel samples" testing. The method may be tested against new samples from known sources, or tested in new areas (as when the method is tested by a new group of investigators in a different geographic region). During this stage of testing, important parameters such as sensitivity, specificity, and temporal and spatial variability may be established. The fourth stage is "comparative" testing: the method is formally compared to other methods and its ability to correctly classify samples is established. This allows methods to be*

ranked according to relative abilities and costs. The fifth stage is testing the method's ability to identify blind proficiency samples. The fourth and fifth stages may occur concurrently. The sixth stage, "proof of the pudding" testing, is application of the source-tracking method in field studies, followed by measurement of the resulting improvement in water quality.

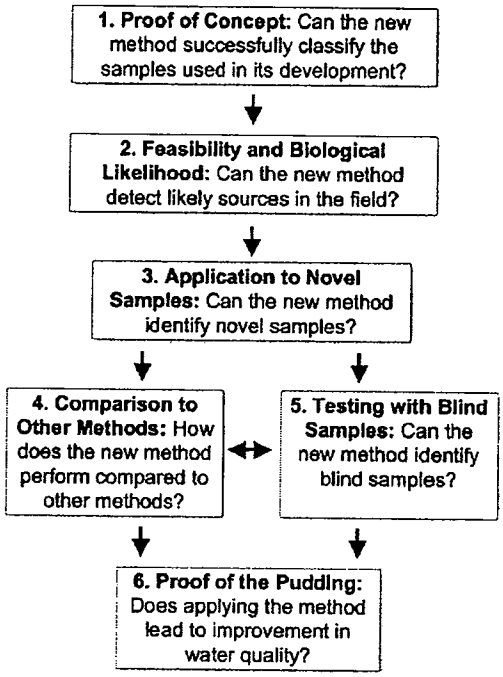


Figure 1. Stages of testing during development of methods of fecal source tracking. Excerpted from Field and Samadpour 2007.

5. The steps undertaken during the identification of the poultry associated biomarker and assay development meet the applicable steps presented above (items number four and six is not applicable to this study). For example, step number one was met by testing samples from IRW that were originally used to develop the method for the presence of the biomarker. Step number two was met by testing samples from throughout the IRW including additional litter (that were not used for developing the method), soil, edge of field run-off water, stream water, groundwater, and lake water. The third step was met by testing non-poultry fecal samples (for the absence of the poultry associated biomarker),

poultry fecal samples (for the presence of the biomarker) and litter samples (for the presence of the biomarker) from geographic regions across the United States including the IRW, Georgia, Minnesota, Colorado, West Virginia, Florida, Utah, Ohio and Idaho. Additionally, the third step was met by blind testing of samples provided to an independent laboratory with the biomarker assay for third party validation of the method. Specifically, the biomarker assay was tested in Dr. Mike Sadowsky's laboratory at University of Minnesota on blind samples provided by North Wind that originated in the IRW. Dr. Sadowski's results correlated with the results generated by North Wind in non-target fecal samples and litter samples. The fifth step was met by testing samples provided as DNA extracts from Dr. Donald Stoeckel and Dr. Marirosa Molina at the USGS and EPA, respectively.

6. The specific methods used in identification of a bacterium associated with poultry litter included polymerase chain reaction (PCR), quantitative PCR (QPCR), terminal restriction fragment length polymorphism (T-RFLP), and cloning. These methods have been routinely used in the development and validation of novel bacterium for a variety of different fecal sources. An abbreviated summary of the number of references using these methods are presented in Table 1. While there are numerous MST manuscripts using these methods, this abbreviated list presents an overview of the different researchers using the specific methods that were used in this study.

7. **Table 1.** Manuscripts using the methods for MST that were utilized in the poultry biomarker study for in the identification of a poultry-litter associated biomarker:

Method	References using this method for MST
PCR	• Bernhard & Field, 2000, Applied and Environmental Microbiology, 66(4): 1587-1594 • Bernhard and Field, 2000, Applied and Environmental Microbiology,66(10): 4571-4574 • Carson et al 2005. Applied and Environmental Microbiology, 71:4945-4949 • Dick et al 2005. Applied and Environmental Microbiology, 71:3184-3191 • Dick et al 2005. Applied and Environmental Microbiology, 71:3179-3183 • Duran et al 2005. Water Research 40:67-74 • Esseili et al 2008. Water Research. 42:4467-4476 • Gourmelon et al 2007. Applied and Environmental Microbiology, 73:4857-4866. • Graves et al 2007. Water Research. 41:3729-3739Hamilton, MJ, T. Yan, MJ Sadowsky, 2005. Applied and Environmental Microbiology, 72(6): 4012-4019 • Haznedaroglu et al 2006. Water Research 41:803-809. • Ivanetich et al 2005. Journal of Microbiological Methods 67:507-526. • Kildare et al 2007. Water Research 41:3701-3715. • King et al 2006. Journal of Microbiological Methods 68:76-81. • Lamendella, et al., 2007. Microbiological Ecology. 59:651-660 • Layton et al 2006. Applied and Environmental Microbiology, 72:4214-4224. • Lu et al., 2003, Applied and Environmental Microbiology, 69(2): 901-908 • Lu et al 2007. Water Research 41:3561-3574. • Mohapatra et al 2007. International Journal of Medical Microbiology 298:245-252. • Okabe, S., Okayama, N., Savichtcheva, O., Ito, T., 2007. Appl. Microbiol. Biotechnol. 74, 890–901. • Ott et al 2000. Journal of Applied Microbiology 91:54-66. • Shanks, O.C., 2006, Applied and Environmental Microbiology, 72(6):4054-4060 • Shanks et al, 2008 Applied and Environmental Microbiology, 74 • Somareli et al, 2007, Journal of Environmental Management, 82 • Ufnar et. Al., 2007 Applied and Environmental Microbiology, Vol 73
qPCR	• Kildare et al 2007. Water Research 41:3701-3715. • Layton et al 2006. Applied and Environmental Microbiology, 72:4214-4224. • Okabe, S., Okayama, N., Savichtcheva, O., Ito, T., 2007. Appl. Microbiol. Biotechnol. 74, 890–901. • Shanks et al, 2008 Applied and Environmental Microbiology, 74
TRFLP	• Bernhard & Field, 2000, Applied and Environmental Microbiology, 66(4): 1587-1594 • Bernhard and Field, 2000, Applied and Environmental Microbiology,66(10): 4571-4574 • Dick et al., 2005, Applied and Environmental Microbiology, 71(6): 3184-3191 • Ufnar et. Al., 2007 Applied and Environmental Microbiology, Vol 73
Cloning	• Bernhard & Field, 2000, Applied and Environmental Microbiology, 66(4): 1587-1594 • Bernhard and Field, 2000, Applied and Environmental Microbiology,66(10): 4571-4574 • Dick et al., 2005, Applied and Environmental Microbiology, 71(6): 3184-3191 • Lamendella, et al., 2007. Microbiological Ecology. 59:651-660 • Layton et al 2006. Applied and Environmental Microbiology, 72:4214-4224. • Lu et al., 2003, Applied and Environmental Microbiology, 69(2): 901-908 • Lu et al 2007. Water Research 41:3561-3574. • Okabe, S., Okayama, N., Savichtcheva, O., Ito, T., 2007. Appl. Microbiol. Biotechnol. 74, 890–901. • Simpson et al, 2004, FEMS Microbiology Ecology, 47 • Ufnar et. Al., 2007 Applied and Environmental Microbiology, Vol 73

8. The biomarker PCR assay is specific to poultry waste (when compared to published specificities of various MST methods and the assay has a known rate of error

based on that specificity. Testing of the poultry waste associated biomarker assay has been ongoing since 2005 to the current day. Hundreds of samples have been tested. Samples tested have included those that should contain the biomarker (poultry litter, chicken feces and turkey feces) and those that should not contain the biomarker (non-target animal feces or feces other than chicken or turkey). The number of samples tested, the types of samples tested, the results of the testing and the geographic region where the samples originated are shown in Table 2. As shown in Table 2, all poultry litter samples tested from the IRW and from another geographic region, namely Georgia, contained the poultry litter associated biomarker. Unused litter did not contain the biomarker. This shows that poultry feces is the source of the biomarker in the poultry litter. The biomarker was found in chicken and turkey feces. The biomarker assay is very specific and was found in only7% of non-target samples, making this biomarker assay is 93% specific when compared to non-target fecal material. This 93% specificity of the assay is the error rate for the assay. The specificity of the poultry waste associated biomarker assay is as good if not better than the accepted specificity of various MST assays that have been published in scientific journals (Figure 2).

9. **Table 2.** Specificity of the poultry litter associated biomarker against target and non-target animal fecal sources and used and unused litter:

Fecal source	Type of sample	Geographic location	Number tested	Number positive
Used Litter	Composite of 18 samples	Oklahoma	10	10
	Composite of 10 samples	Georgia	7	7
Unused Litter	Duplicates	Oklahoma	3	0
Chicken	Composites of 10 broiler scats	Georgia	2	2
	Individual fecal swabs of layers	Florida	7	3
	Composite of layer fecal slurry	Florida	5	4
	Individual fecal swabs of layers	Minnesota	5	3
	Composite of 10 layer scats	Utah	4	2

Turkey	Individual Fecal swabs	Minnesota	2	1
Beef cow	Duplicate composites of 10 pats	Oklahoma	9	0 [a]
	Duplicate composites of 10 pats	Arkansas	6	0 [a]
	Composite of 10 pats	Colorado	1	0 [b]
Dairy cow	Duplicate composites of fecal slurries	Missouri	3	0 [a]
	Duplicate composites of fecal slurries	Oklahoma	3	0 [a]
	Individual pats	Florida	10	0 [b]
	Individual pats	Minnesota	6	0 [a]
Swine	Duplicate composite of fecal slurries	Arkansas	2	0 [a]
	Composite of fecal slurries	Missouri	1	0 [a]
Duck	Duplicate composites of 10 scats	Oklahoma	3	1 [a]
	Duplicate composites of 10 scats	Arkansas	2	0 [a]
	Individual scats	Florida	8	0
Goose	Duplicate composites of 10 scats	Oklahoma	3	1 [a]
	Duplicate composites of 10 scats	Arkansas	2	0 [a]
	Individual scats	Idaho	10	1
	Individual scats	Florida	10	1
Human	Septic system	Florida	1	0
		Colorado	1 [b]	0
		Oklahoma	3	0 [a]
	WWTP Influent	Florida	5	0
		West	3 [b]	1
		Virginia	2 [b]	0
		Ohio	5	1
		Minnesota	1	0 [a]
		Oklahoma	2	0 [a]
		Arkansas	9 [b]	2
	WWTP Effluent	Florida	1	0
		Minnesota	2	0
		Idaho	2	0

[a] All non-target fecal samples were non-detect in blind samples provided to Dr. Michael Sadowski's laboratory by North Wind.
[b] Samples provided as DNA extracts to North Wind by Drs. Donald Stoekel and Marirosa Molina of the USGS and USEPA

10. There are not "Applicable Statistical Standards" for MST studies. Evenson et al. 2006, states directly "to date, there is no established standard of precision available for the MST methods." (Carrie J. Evenson a, K.A. Strevett, 2006, Discriminant analysis of fecal bacterial species composition for use as a phenotypic microbial source tracking method, Research in Microbiology 157:437–444) Rather an MST method that is purported to be specific to one animal host is tested against non-target animal fecal samples and then an average relative correct classification (ARCC) or specificity is calculated for the method. In a review of the published ARCC or specificity of various

MST methods it is obvious that the scientific community and regulators views a particular method as begin specific to a particular animal host with only a 60 to 70% correct classification rate. (Harwood, VJ, J Whitlock, V Withington, 2000, Classification of Antibiotic Resistance Patterns of Indicator Bacteria by Discriminant Analysis: Use in Predicting the Source of Fecal Contamination in Subtropical Waters, Applied and Environmental Microbiology, 66(9): 3698–3704).

11. In reviewing the MST literature there are a wide range of ARCC or specificity of methods accepted for various MST methods. A summary of the range in specificity of various MST methods reported in the literature along with the number of samples tested is presented in Figure 2. It should be noted that the number of samples tested during the development of the poultry waste biomarker assay is greater than that used by a majority of the researchers and the biomarker assay has a greater sensitivity that most of the other methods in the literature:

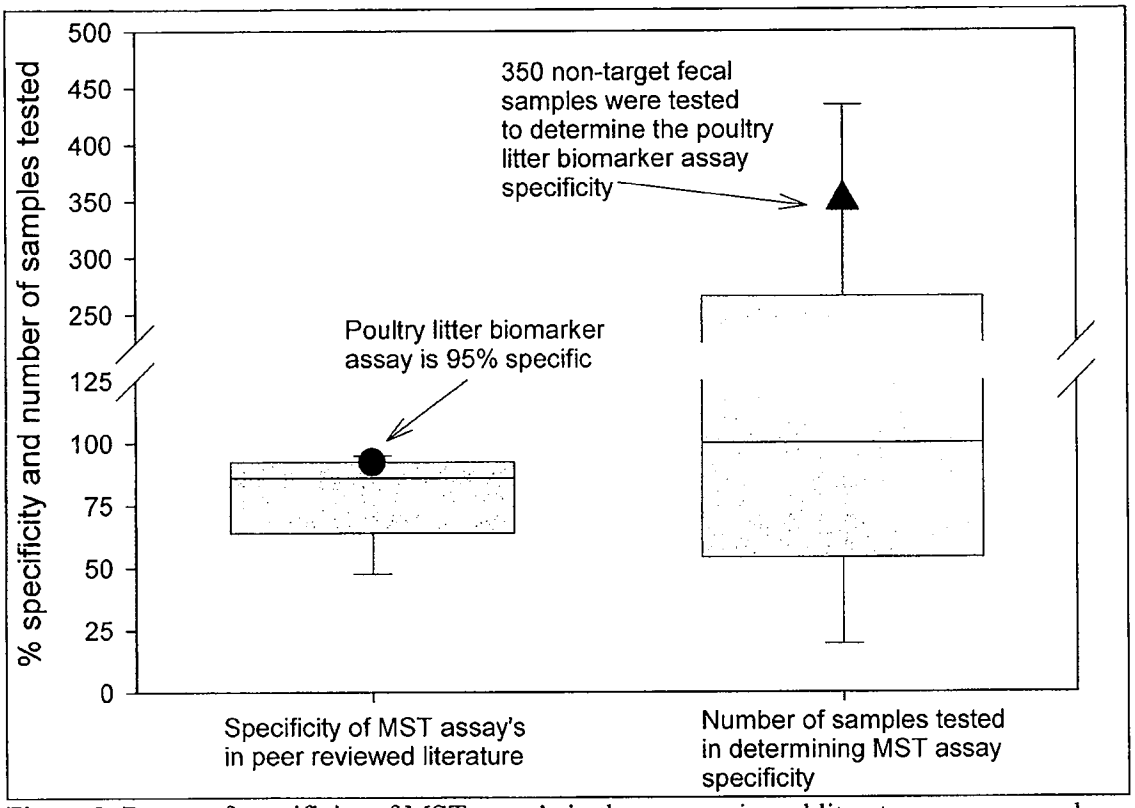


Figure 2. Range of specificity of MST assay's in the peer reviewed literature as compared to the poultry litter associated biomarker (blue circle) and the number of samples tested in MST assays to determine specificity as compared to the number tested for the poultry litter associated biomarker assay (red triangle). [Carson et al 2005. Applied and Environmental Microbiology, 71:4945-4949; Dombek et al. 2000. Applied and Environmental Microbiology, 66(6) 2572-2577; Evenson, C.J., K.A. Strevett. 2006. Research in Microbiology. 157:437-444; Graves et al 2007. Water Research. 41:3729-3739; Kildare et al 2007. Water Research 41:3701-3715; Layton et al 2006. Applied and Environmental Microbiology, 72:4214-4224; Mohapatra et al 2007. International Journal of Medical Microbiology 298:245-252.]

12. This is the method employed for detection and quantification of the poultry biomarker is SYBR green qPCR. SYBR qPCR has been shown to distinguish even one base pair difference (the smallest difference possible) between two DNA sequences in some cases. This type of qPCR method is so reliable in fact that it is routinely used in medial microbiology and diagnostic testing for different human diseases, where the magnitude of an incorrect diagnosis is significantly of greater concern than in microbial

source tracking. (Pals, G. et al, 2001, Detection of a single base substitution in a single cell using the LightCycler™, J. Biochem. Biophys. Methods, 47:121-129) (James F. Papin, Wolfgang Vahrson, and Dirk P. Dittmer, 2004, SYBR Green-Based Real-Time Quantitative PCR Assay for Detection of West Nile Virus Circumvents False-Negative Results Due to Strain Variability, Journal of Clinical Microbiology, 42:4, 1511-1518) (Ririe, KM., et al. 1997, Product Differentiation by Analysis of DNA Melting Curves during the Polymerase Chain Reaction, Analytical Biochemistry, 245: 154-160.).

13. Additional testing was also conducted to determine if the biomarker assay was sensitive enough to detect differences between closely related DNA sequences to the poultry litter associated biomarkers DNA sequence. In particular, thirteen closely related *Brevibacterium* spp. isolates and *B. avium* were isolated from goose feces from Washington and used poultry litter from Missouri. Isolates were provided by the Institute for Environmental Health (IEH) Laboratories. Testing conducted on these isolates included 1) attempted amplification by the qPCR assay, 2) comparing the SYBR melting curve of qPCR-positive isolates to that of the poultry litter associated biomarker and 3) sequencing the 16S rRNA gene to determine the number of differing base pairs between the closely related *Brevibacterium* spp. and the poultry litter associated biomarker. DNA from only nine of the 13 isolates was amplified. Additionally, DNA from those closely related isolates that amplified with the biomarker assay produced a unique melt curve that could be discriminated from the biomarker melt curve (> 1°C difference in peak melting temperature from the LA35 peak melting temperature). DNA sequencing of the closely related isolates revealed only a 5 base pair difference among the 530 base pair DNA product reproduced by the SYBR qPCR between the poultry biomarker and other closely

related DNA sequences. Therefore this assay was sensitive enough to distinguish between the poultry litter biomarker and closely related organisms that varied by in DNA sequence by only 0.9%.

14. In addition to the specific testing of the sensitivity of the melt curve analysis, North Wind's Standard Operating Procedure for analysis of samples for the poultry litter associated biomarker removes all question regarding the "potential error rate" suggested by the Defendants (NWI, 2008, Standard Operating Procedure, Quantification of a poultry specific Brevibacteria biomarker in environmental samples, Revision 2, North Wind, Inc.). While chemicals other than DNA can influence how a melt curve will look by SYBR qPCR, it is very easy to control this potential error in the analysis by including matrix spiked samples. North Wind's procedure follows and actually is more stringent than the EPA's Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples where a matrix spike sample is only recommended for each type of sample tested (e.g., one matrix spike for soil samples, one for water samples) (EPA, 2004, Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples, Office of Water, EPA 815-B-04-001). In North Wind's procedure a matrix spike is included for each sample tested, not just for each general group of samples tested.

15. Third party validation of the biomarker assay was conducted by independent, blind testing of the poultry waste associate biomarker by Dr. Mike Sadowsky's laboratory at University of Minnesota to validate that the that biomarker assay is capable of being tested by the scientific community and that it can be accurately reproduced. North Wind provided samples (i.e., DNA extractions so that the researchers could not identify the

material visually) to the University of Minnesota that had originated from the IRW, and were relabeled with non-descriptive designations so that the samples were truly blind. Dr. Sadowsky's results correlated with the results generated by North Wind in non-target fecal samples and litter samples, confirming that the assay is capable of 1) being tested by other scientists, 2) detecting the biomarker that is associated with poultry waste, and 3) is not found in non-target fecal samples collected from the IRW.

15. Dr. Samuel Myoda's Expert Report claims that he was able to isolate the biomarker sequence from unused bedding material, goose flop, sand beach frequented by geese, other water fowl samples and cow hide samples. In reviewing the laboratory notes, deposition testimony, and raw data used to come to these conclusions there are serious doubts about the validity of these claims as follows:

1. IEH and Dr. Myoda's technicians did not follow Regulatory guidance and standard scientific protocol for construction of their laboratory space;

2. IEH and Dr. Myoda's technicians did not follow Regulatory guidance and standard scientific protocol for negative controls for DNA extractions, PCR and qPCR;

3. IEH did not actually test the biomarker assay as they were unable to get the qPCR method to work. However Dr. Sadowsky's laboratory was able to use the qPCR method successfully indicating the method is able to be tested;

4. When IEH could not reproduce the biomarker assay they used a less sensitive method that has a higher error rate than the qPCR method for analysis of samples;

5. IEH repeatedly violated their own QA/QC guidelines for analysis of the samples for the biomarker assay; and

6. There is evidence to suggest the samples collected independently by the defendants and supplied to IEH for analysis were contaminated with poultry litter.

16. The EPA's Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples (EPA, 2004, Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples, Office of Water, EPA 815-B-04-001) recommends the following for laboratories performing PCR:

> *"The high sensitivity of PCR techniques requires that demanding assay conditions be followed. The laboratory should be designed and operated in a way that prevents contamination of reactions with amplified products from previous assays and cross-contamination between samples, both of which can lead to false-positive results... Contamination between samples and from previous PCR amplicons generated in the laboratory is a significant potential source of invalid PCR results. Thus, the separation of work space is critical. A laboratory performing PCR analyses on environmental samples should be divided into at least three physically separate rooms:*
>
> - *Reagent preparation (using positive pressure to prevent the introduction of contamination)*
> - *Sample preparation (using negative pressure to keep template nucleic acids in the room)*
> - *Amplification and product detection (using negative pressure to keep amplified nucleic acids in the room)"*

As shown in the Exhibit 2 from Myoda's deposition (Figure 3 below), his laboratory space was not constructed in such a manner as to prevent the cross-contamination of samples during testing. Specifically in the center of the drawing, labeled middle by Myoda, he clearly indicates that DNA extractions, a PCR area or thermocylcers, gel setup and Mike's (one of Myoda's technicians performing bacterial culturing) lab area all used shared laboratory space that was physically adjacent to one another. This co-location of these types of activities violates the EPA recommendations for keeping "nucleic acids" or DNA sample preparation areas physically separate from the area where "amplification and product detection" or PCR and gel setup occurs.

The potential for cross-contamination calls into question the validity of the entirety of the PCR and qPCR results generated by his laboratory.

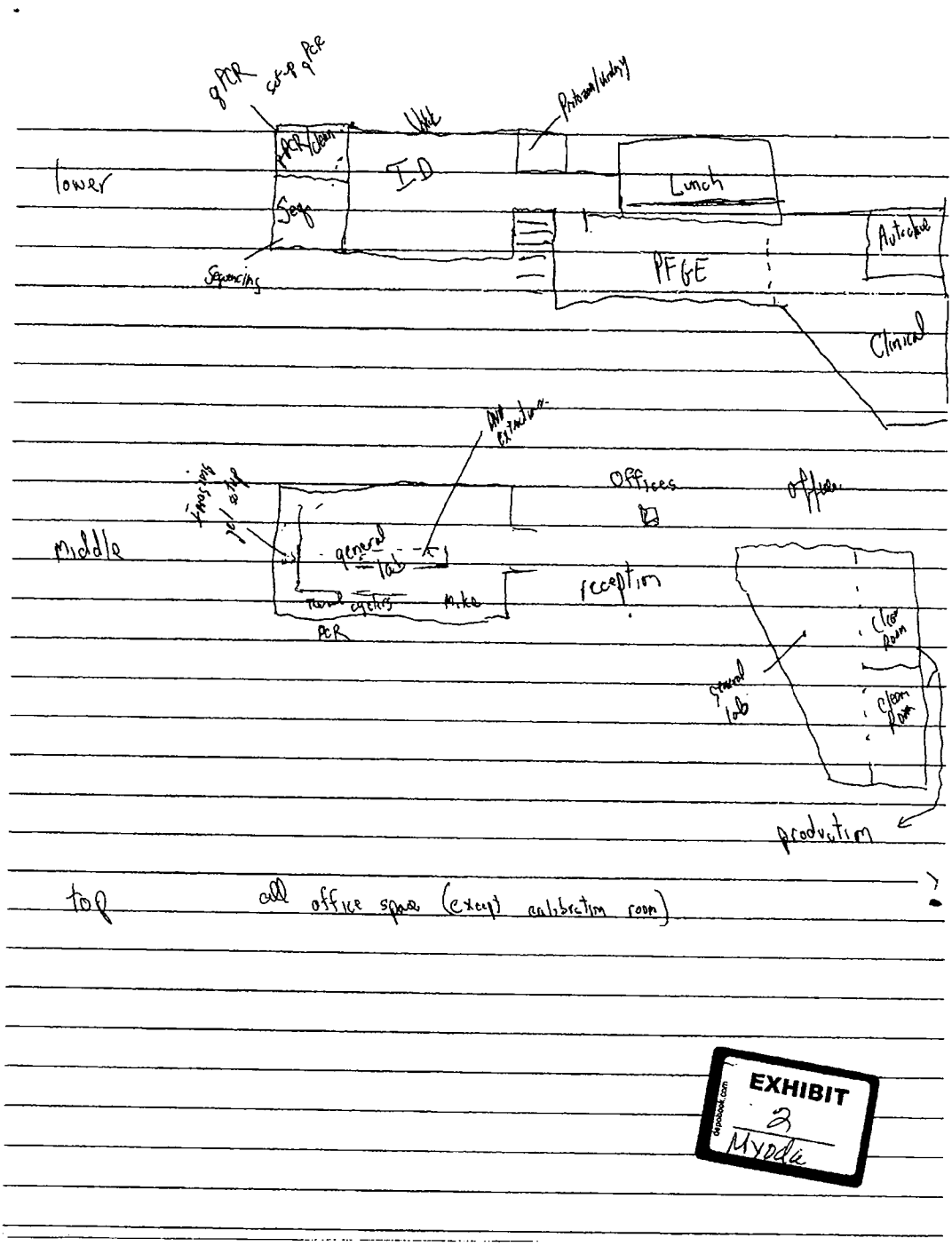


Figure 3. Layout of the Dr. Myoda's laboratory space at IEH.

17. The EPA's Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples (EPA, 2004, Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples, Office of Water, EPA 815-B-04-001) recommends the following method blank or negative control sample:

> *The method blank is designed to check for contamination throughout sample processing and PCR analysis. This control is performed on a sterile reagent water sample that is processed with the test samples using the same preparation, extraction, sample transfer, and PCR procedures as the test samples. At a minimum, method blank samples should be performed once per batch. A sample batch is defined as a set of test samples processed together through all steps of the method leading to PCR.*

The method blank controls for cross-contamination of samples and is introduced in the process beginning with DNA extraction from the primary sample. For instance in IEH's analysis they would need to include a DNA extraction negative control for each group of samples analyzed (i.e., unused bedding material, goose flop, sand beach frequented by geese, other water fowl samples and cow hide samples). However, there is no mention of DNA extraction negative controls in the entire submittal by IEH that was used as the basis for Dr. Myoda's conclusions regarding the biomarker being present in these non-target samples. As there were not any method blank samples (i.e., DNA negative extraction controls) included in Dr. Myoda's analysis in addition to having a laboratory layout that is known to greatly increase the possibility of cross-contamination there is serious doubt as to whether the environmental samples, water and goose feces actually contained the biomarker or if the samples were contaminated during sample processing.

Lack of inclusion of the appropriate negative controls (i.e., DNA extraction negative controls) is especially troubling as noted in Dr. Myoda's considered materials that on 3 separate occasions one of Dr. Myoda's technicians described in his laboratory notes contamination in their culture media or blank culture controls [Myoda-Samadpour-0000059, Myoda-Samadpour-0000063, Myoda-Samadpour-0000064]. As Dr. Myoda depended on enrichments, or growing the microorganisms from very small numbers in the original samples to many million microorganisms over time, there is serious question about the ability of his technicians to keep the samples free from contamination. An appropriate negative control could have accounted for this possible error, however this step was not performed based on a review of the entirety of the materials Dr. Myoda depended on to make reach his conclusions regarding the validity of the biomarker assay.

18. Dr. Myoda's technicians did not replicate the biomarker assay as the IEH laboratory conducted primarily PCR testing using the biomarker specific primers. PCR and qPCR specificity is actually significantly different and the additional sensitivity of the qPCR SYBR melt curve analysis is required to determine if the assay is detecting the poultry litter associated biomarker or not. For example, as Papin et al explain:

> *"...since the readout in conventional or nested PCR is a band on a 2% agarose gel that can differentiate only gross variations in size (>5 bp, depending on the gel system) and not nucleotide substitutions. In contrast, the SYBR green-based assay provides an amplicon dissociation profile as an added measure of specificity. With this method, even single nucleotide changes can yield an altered profile, which should allow for the rapid routine detection of sequence variations." (Papin, J.F., et al, 2004, SYBR Green-Based Real-Time Quantitative PCR Assay for Detection of West Nile Virus Circumvents False-Negative Results Due to Strain Variability, J. Clinical Microbiology, 42(4):1511-1518.)*

As stated by Papin, the qPCR melt curve analysis is significantly more sensitive than PCR analysis using gel electrophoresis which was heavily used by Dr. Myoda in drawing

his conclusions. In the few instances where Dr. Myoda and his technicians did use qPCR they did not include appropriate controls (i.e., negative DNA extraction controls) and what controls were included indicated that they had contaminated samples as the negative qPCR control had a melt peak indicative of the control sample containing the biomarker. The only way a negative qPCR control would have a melt peak profile indicative of the biomarker is if there was contamination of the qPCR plate or chemical reagents during handling. Contamination of the negative qPCR control invalidates all results for that analysis. Dr. Myoda used the qPCR results from this run with known contamination as his only proof that the cow hide samples contained the poultry litter biomarker.

19. IEH collected material directly for this project in direct violation of the QA/QC manual. The MEI/IEH QA/QC manual states [Myoda 0003721]:

> *"MEI/IEH does not conduct product sampling rather receives samples from customers. Environmental samples for microbiological analysis, are sometimes collected on customer's premises by MEI/IEH representatives, and are taken according to generally accepted practice."*

The laboratory notes that Dr. Myoda relied on in generating his opinion regarding the validity of the poultry litter associated biomarker assay indicate that on 2 separate occasions, his laboratory assistants collected samples directly for this case (in particular goose feces and beach sand) from Washington State [Myoda 3648 and 3687, see attached]. MEI/IEH does not have a QA/QC protocol for environmental sample collection as indicated by the list of QA SOPs in the QA/QC manual [Myoda003724, Myoda003725 and Myoda003726, see attached]. As MEI/IEH has a standard rule against collection of samples by laboratory personnel and they do not have procedures in place guiding the collection of those samples there is some question as to whether the

appropriate methods were used for collection of environmental samples to prevent cross contamination of the samples.

20. Dr. Myoda determined that the poultry litter biomarker was found in unused litter material. However, these materials were likely contaminated with the poultry litter associated biomarker as the samples were collected from after the litter material had been spread in a poultry barn that had previously contained poultry, but before the birds were placed on the unused litter material (Myoda-Samadpour-0000160, see attached). Gene's Simmons affidavit states:

> *"On July 22, 2008, I retrieved a sample of clean bedding from a poultry barn located on Farm 25...The bedding was clean and unused; no chickens had been placed on the bedding prior to July 22, 2008...I obtained the sample by using an 8-ounce clean and unused Styrofoam cup and scooping it into the bedding and then pouring the cup of bedding into a plastic bag. I scooped bedding from 4 to 5 different places inside the house."*

Even though poultry had not been placed on the unused litter it is highly likely that unused litter material was contaminated during 1) mechanical spreading of the litter material in the barn that had previously contained poultry, and 2) exposure to the floors, walls and bacteria in the air at within the barn which may harbor some low concentration of the biomarker for a period of time after poultry are removed. As the unused poultry litter was likely exposed to the poultry biomarker before sampling, these samples do not represent a litter material that has not been exposed to poultry. Therefore it is not surprising that Dr. Myoda's laboratory found the poultry biomarker at very low concentrations in the litter material. Additionally, a negative DNA extraction control was not included with these samples, therefore there is a likelihood that cross-contamination could have occurred during sample processing in Dr. Myoda's laboratory.

I declare under penalty of perjury, under the laws of the United States of America, that the foregoing is true and correct.

Executed on the 26th day of May, 2009.

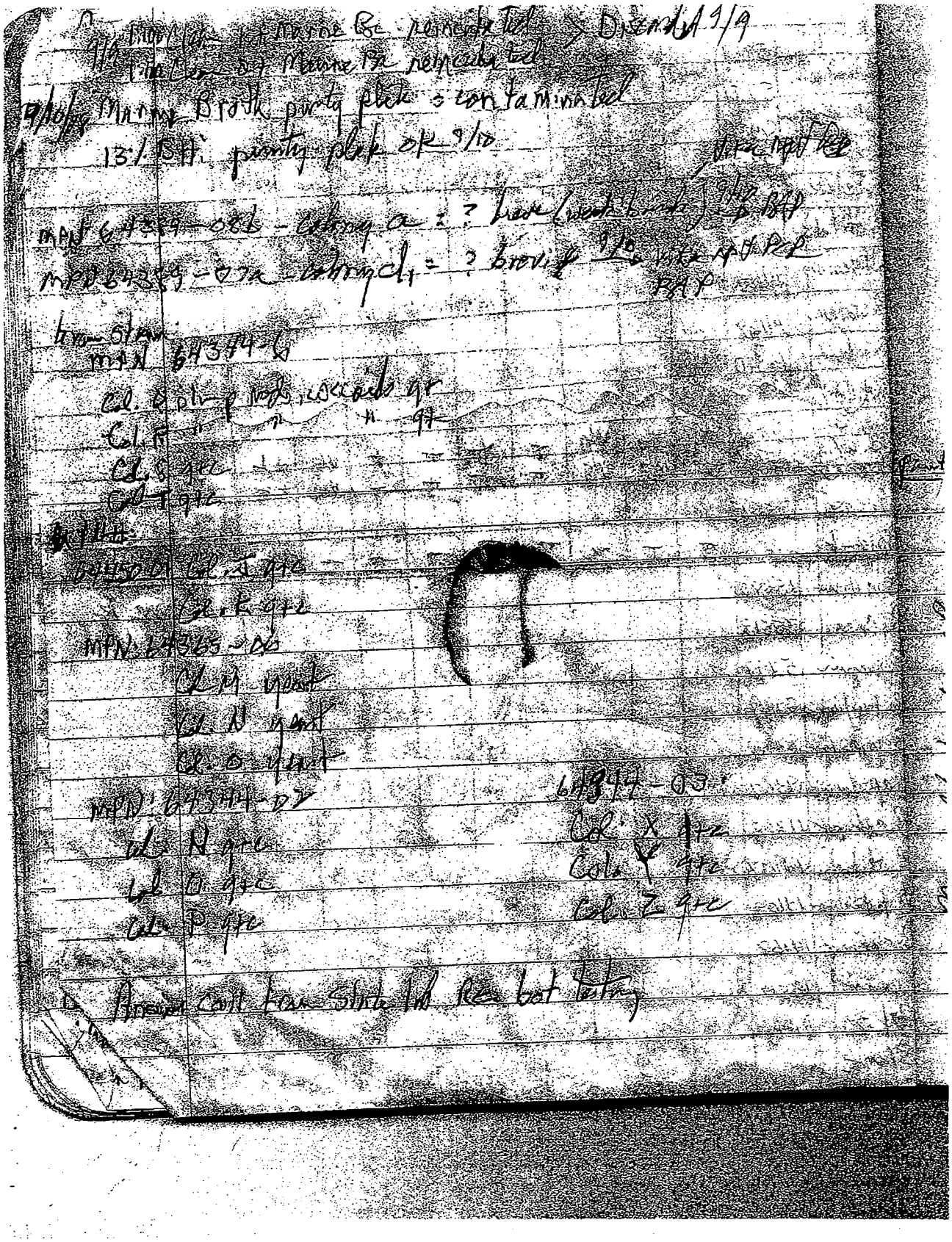
JENNIFER L WEIDHAAS, Ph.D., P.E.

ATTACHMENTS

9/3/08 0810 [illegible]
made [illegible] [illegible]
gave 5 plates to Vika for PCR
10:20 - Break
10:35 Worked on [illegible] paper revision for Greg [illegible]
11:00 Greg suggested King MYP plate to our last Brevi-[illegible]
From this → innoc Clean 1A, 1B, 2A, 2B, dirty
Clean 1A, BH [illegible] (old) → MYP
12:10 Lunch
1:10 - [illegible] salt tolerance 24 hr culture
- some contamination → TSA plate
[illegible] everything re-[illegible]
3:00 talked to Anton re contamination in Tinsdale
3:30 - 3:40 - Break
3:40 → 4 pm - catch up [illegible] lab notebook
4:15 record Brevi time for today [illegible] 9/4/08
9/4/08 [illegible] 48h salt tolerance [illegible] third plate much contamination
test discarded contaminated [illegible] B. cereus
[illegible] 0%, 5%, 10%, 15% [illegible] only [illegible]
of Brevi, [illegible] inoculum. If no contam - [illegible]
10:00 Break in 1% increments
12:40 - make up 2x Tinsdale [illegible] (15.4 gm)
2x batch tinsdale - 16 pl [illegible] 14 ml/plate
Re [illegible] - re-ordered PEA base 500 gm # 454302

NaCl	#	0	1%	3%	5%	7%	9%	11%	13%	15%	NaCl Tol
B. sanguin	5797										
B. linnyiodin	5799										
B. pancivbtar	8470										
B. epidermidi	9307										
B. celere	11203										
B. otitidis	14107										
B. luteolum	14109										
B. stationis	14168										
B. iodinum	18510										
B. [illegible]											
Col. 3	Dirty	+	vlg	vlg	+	+	+	+	vlg	+L	
B. avium	15880	+	vlg	vlg	+	+	+	+	vlg	+L	
B. casei	35513										
Blank	-	-	+	-	-	-	-	-	-	-	
B. avium	15880										Marine [illegible]
B. casei	35513										
B. sanguin	5797										
B. linniodin	5799										
B. pancivbran	8470										
B. epidermidi	9307										
B. celere	11203										
B. otitidis	14107										
B. luteolum	14109										
B. stationis	14168										
B. iodinum	18510										
Col 3	Dirty										
Blank											

Myoda-Samadpour-0000063



Myoda-Samadpour-0000064

STATE OF MISSOURI)
) ss.
COUNTY OF McDonald)

AFFIDAVIT OF GENE SMITH

Comes now the affiant and states as follows:

1. I am employed by Simmons Foods, Inc. as the broiler manager of company managed farms.
2. On July 22, 2008, I retrieved a sample of clean bedding from a poultry barn located on Farm 25, which is a company managed farm located approximately 3 miles east of Simmons Foods, Inc.'s Southwest City, Missouri facility. The bedding was clean and unused; no chickens had been placed on the bedding prior to July 22, 2008.
3. I obtained the sample by using an 8-ounce clean and unused styrofoam cup and scooping it into the bedding and then pouring the cup of bedding into a plastic bag. I scooped bedding from 4 to 5 difference places inside the house.
4. I placed the bedding I scooped into a clean and unused plastic bag. I then sealed the bag with tape. I then filled another clean and unused plastic bag with clean bedding using the same steps I used with the first bag. I then sealed the second bag with tape.
5. On that same day I used the same method to obtain samples of used bedding in a house on Farm 26. Farm 26 is a company managed farm located approximately 3 miles east of Simmons Foods, Inc.'s Southwest City,

Juanita Beach is located at North End of lake Washington

11/17/08 4:45pm Collected Juanita Beach sand (under goose prints)
" " " loam/soil (under goose feces - lots of goose feces!)

11/20/08 PM Vika ran PCR on ich soil x2 she collected
- negative for Brevi
" Vika ran Juanita Beach soil/feces - neg for Brevi
" Vika ran Juanita Beach Sand - Pos for Brevi by PCR
" Reg Sand → 0.1% PW → BAP, TSA, MRS + Van MRS

AM 11/21/08 MRN Hide Samples + for Brevi by PCR → 65019-03 → & 65019-01 →
PEA, MRS, MRS+Va

Sun 11/23/08 2:45 Picked 65019-01, 03 → Pl. PEA (Batch 6907)
multiple colonies picked, orig. plates → Window @ RT
Picked Juanita Beach Sand / Soil → PEA (multiplied.)
orig. pl. → window (RT)

Mon 11/24/08 BATCH 6924 - PEA + Vancomycin (0.12g/L)

11/25 Juanita Beach - Beach (Sand) - BAP, PEA, MRS very heavy gr. 11/25
Juanita Beach - grass/soil/goose - no bands compatible w Brevi found
no further testing.
Gram stains col picks from 11/23/08 (Sunday AM)

Hide Sample 65019-1:
(Sponge)
- MRS col a: g+ plus rods, cocco bacilli 11/25 → PCR
- MRS col b: g+ rods, cocco bacilli 11/25 → PCR
- MRS col c: g+ plus rods, long rods unpaired, 11/25 → PCR

Myoda003648

8/1/08 Sunny, ≈78°F

(A) gull @ Kenmore log boom
brn striped pin feathers

Swabbed c̄ 3 cotton tipped swabs into whirlpak

(B) Fresh Goose @ Green Lake
grass, across from boat rental launch, near "feeding bay"

(C) Goose @ Green lake
grass meadow further NE of "B"

Refrigerated [signature] 8/1/08

8/3/08 7:30–8:30 pm

goose 1 Juanita Beach
2 Grassy area between Picnic
3 Shelter & ~~Life~~ (former) Life
4 guard stands at beach.
5
6

7 grassy area midway in
8 Park, near snack stand;
9 grove of trees.
10
11
12
13
14

[signature] 8/4/08

[redacted]

2.6.3.3 Intermediate checks of reference standards and materials are conducted according their individual SOPs or EPOIs.

2.6.3.4 MEI/IEH assures the safe handling, transport and storage of reference standards and materials according to SOP IWQA017 and the specific standard's SOP or EPOI.

2.7 Sampling

2.7.1 MEI/IEH does not conduct product sampling rather receives samples from customers. Environmental samples for microbiological analysis, are sometimes collected on customers' premises by MEI/IEH representatives, and are taken according to generally accepted practice. Product sub-samples may be taken in the laboratory according to sample preparation instructions in specific test SOPs.

2.8 Handling of Test and Calibration Items

2.8.1 MEI/IEH receives handles and stores samples in a manner to prevent loss of integrity. Procedures for sample receipt and handling are defined in SOPs IWGN018 and IWGN025.

2.8.2 MEI/IEH identifies each sample with a unique number generated by the Laboratory Information Management System (LIMS) software.

2.8.3 Log-in personnel record any sample abnormalities on the associated worksheets. If there is any question about the suitability or description of the sample, the customer is consulted prior to beginning analysis. The customer's instructions are recorded.

2.8.4 MEI/IEH has appropriate storage facilities to avoid deterioration of samples. Sample retention and disposal procedures are defined in SOP IWGN025.

2.9 Assuring the Quality of Test and Calibration Results

2.9.1 MEI/IEH analyzes quality control samples daily for each test that it performs. Also, control samples are run for each batch of tests. Control charts are constructed for these samples and are monitored on a frequency appropriate to the analysis. Appropriate corrective actions are taken when trends or out-of-control situations are identified.

2.9.2 MEI/IEH participates in proficiency sample programs where available. These programs are administered by outside commercial entities or government

- **General Standard Operating Procedures:**

Item No.	Document Description	Document Number
1	Receiving, Documenting, and Resolving Customer Complaints	GN004
2	Equipment Performance, and Operating Instructions-Structure, Format, Implementation, and Use	GN005
3	Standard Operating Procedure (SOP) Format Guidelines	GN006
4	Test Methods-Structure, Format, Implementation, and Use	GN007
5	Procedure for Purchasing	GN008
6	Control and Maintenance of Documents	GN010
7	Basic Rules of the Lab	GN011
8	Building Security	GN012
9	Variability and Bias Calculation	GN013
10	Precluding Conflicts of Interest	GN014
11	Protection of Confidential Information and Proprietary Rights	GN015
12	Traceability to IEH-MEI Standards, References, and Standard Reference Materials	GN016
13	List of Tests Run at IEH/MEI	GN017
14	Receipt of Samples	GN018
15	Contract Writing and Review	GN021
16	Record Keeping	GN022
17	Preventive Action	GN023
18	Receipt and Storage of Reagents and Laboratory Consumables	GN024
19	Storage and Disposal of Laboratory Samples	GN025
20	Handling and Discard of Compromised Samples	GN026
21	Disposal of Chemicals, Reagents and Equipment	GN028
22	Laboratory Cleaning	GN029
23	Control the use of the term "A2LA" and "A2LA Accredited" symbol	GN030
24	Pest and Vector Control	GN032
25	Emergency Preparedness and Response Plan	GN033
26	Guidelines for CLIA Specific Documentation	GN034
27	Packaging and Shipping Category A Biological Specimens	GN035
28	Packaging and Shipping Category B and Exempt Biological Specimens	GN036
29	Privacy and Security of Health Information, HIPAA	GN056

● **Quality Assurance Standard Operating Procedures:**

Item No.	Document Description	Document Number
1	Assignment and Tracking SOP Numbers	QA001
2	IEH/MEI Training Program	QA003
3	Tracking of Critical Lots of Materials Used in Testing	QA006
4	Review of Incoming Work for Adequacy of Facilities and Resources	QA007
5	Internal Audits and Reviews	QA008
6	Management Review of QA System	QA009
7	Proficiency Programs Participation	QA010
8	Use of Feedback and Corrective Action with IEH/MEI SOPs, TMs, and EPOIs	QA011
9	Departing from Documented SOPs (SOPs, TMs, and EPOIs)	QA012
10	Run Rules for IEH/MEI Control Charts	QA013
11	Responsibility and Authority and Interrelationships of key IEH/MEI Personnel	QA014
12	Root Cause Analysis Procedure	QA016
13	Reference Material Handling	QA017
14	Estimating Measurement Uncertainty	QA018
15	In-House Calibration of Balances, Thermometers, and Volumetric Pipettes	QA019
16	Access to Data Entry and Issuing Reports	QA020
17	Analytical Data Review	QA021
18	Calibration and Verification of Temperature Controlling Equipment	QA022
19	Validation of Developed Computer Software	QA038
20	Computer Validation	QA039
21	Cleaning of Laboratory Glassware	QA040
22	Control of OOS Results	QA046
23	Change Control System	QA047

● **Test Methods**

Item No.	Document Description	Document Number
1	Sample Preparation for Microbial Analyses	MB001
2	Coliform/*E. coli* Count - Petrifilm	MB072
3	Aerobic Plate Count – Petrifilm	MB078
4	Enumeration of Generic *E. coli* by IEH-MPN Method	MB081
5	Detection of *Salmonella* in Meat and Environmental Samples	MB094
6	Detection of *E. coli* O157:H7 by Multiplex PCR	MB217
7	Detection of EHEC/STEC by Multiplex Method	MB 218
8	Detection of *Salmonella* in Food Samples by Multiplex Method	MB315
9	Detection of *Listeria* sp. and *L. monocytogenes* by Multiplex Method	MB316
10	Pulsed Field Gel Electrophoresis (*E. coli* O157:H7, *Salmonella* L. monocytogenes)	MB331
11	Microbial Source Tracking (MST) by rRNA Typing	MB333
12	Lactic Acid Bacteria Count - Petrifilm	MB343
13	Bacterial Subtyping by Micro Restriction Fingerprinting (MRF)	MB431
14	Enumeration of *Enterobacteriaceae* - Petrifilm	MB444
15	Acridine Orange Direct Count: Total Bacterial Count (Epifluorescent Bacterial Count)	MB448
16	Identification of Microorganisms by DNA Sequencing	MB450
17	Bacterial Subtyping by Pulsed Field Gel Electrophoresis	MB454
18	Microscopic Analysis of Microorganisms	MB457
19	Phenotypic Analysis of Microorganisms	MB473
20	*Bacillus* Diarrhoeal Enterotoxin Visual Immunoassay	MB476
21	Yeast and Mold Count - Petrifilm	MB492
22	Detection of *Bacillus cereus* Enterotoxin L_2 by Using the BCET- RPLA® Diagnostic Kit	MB496
23	Detection of *Bacillus cereus* Group Toxins by PCR	MB505